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TITLE: Vitamin D3 and Vitamin D3 Analogs as Protectants Against the Cardiotoxicity of Chemotherapeutic Agents Utilized in the Treatment of Breast Cancer

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14. ABSTRACT
Studies were performed to determine whether 1,25 dihydroxy vitamin D, the active form of this hormone, could protect cardiomyocytes from the toxicity of chemotherapy and radiation. Studies were performed in two models of cardiomyocyte function, H9c2 and HL1 cardiomycoytes. However, 1, 25 dihydroxy vitamin D3 failed to protect against either radiation, adriamycin (doxorubicin) or paclitaxel. We also tested sildenafil as a cardioprotectant, as this phosphodiesterase-5 inibitor has been shown to suppress ischemia –reperfusion injury to the heart. However, neither sildenafil alone nor sildenafil in combination with vitamin D demonstrated significant protection. Subsequent studies indicated that the toxicity of radiation may be related to the promotion of autophagy. In the course of this work, we developed an animal model of radiation induced cardiac injury and studies are ongoing to evaluate other cardioprotective strategies, such as the use of IL-1.

15. SUBJECT TERMS
autophagy, breast cancer, MCF7, irradiation, vitamin D, doxorubicin, paclitaxel

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INTRODUCTION:

Doxorubicin (Adriamycin) and Trastuzum ab (Herceptin) as well as ionizing radiation are all utilized for the treatment of breast cancer. Doxorubicin therapy is constrained by the developm ent of cumulative dose-limiting cardiomyopathy; taxanes have been shown to exacerba te the cardiotoxicity of doxorubicin while Herceptin therapy is also associated with cardiotoxicity , alone and in combination with either doxorubicin or the taxanes (1). Radiation administered during treatment of breast cancer results in cardiac damage that is often delayed for years. Vitamin D_3 receptors have been identified in the hum an heart (2) and Vitam in D_3 has been shown to contribute to improved heart function (3). We had proposed that Vitamin D_3 and/or less calcemic analogs could be effective in protection of the heart against the toxicity of chemotherapy and radiotherapy.

BODY:

It should be noted that we have been unable to obtain the vitamin D analog, EB 1089, that was used in much of our previous work due to the fact that the license was purchased from Leo Pharmaceuticals in Denmark by Cougar Inc in California, while Cougar was subsequently acquired by Johnson and Johnson. We had submitted a materials transfer agreement almost two years ago; however, the company has not acted on it. Consequently, our research efforts were focused solely on 1,25 dihydroxy vitamin D3, the natural active form of the hormone.

In the studies presented in Figure 1, we evaluated the effects of vitamin D on the sensitivity to radiation in HL-1 cardiac myoctes at radiation doses of 5 Gy and 10 Gy. It is evident that Vitamin D alone provided no protection in this experimental model system. In view of data in the literature indicating that the phosphodiesterase inhibitor sildenafil (Viagra) had cardioprotective actions (4), we investigated the impact of sildenafil alone and in combination with Vitamin D. However, this combination also proved to be ineffective in conferring protection.

In the studies presented in Figure 2, similar experiments were performed utilizing HL-1 cardiomyocytes exposed to various concentrations of adriamycin (doxorubicin) in the presence and absence of vitamin D/sildenafil. As in the studies presented in Figure 1, we were unable to demonstrate any degree of protection.

Similar studies to those shown in Figure 2 were performed utilizing another cardiomyocyte cell line, H9c2 cells, that have been used for many years as a model of doxorubicin induced cardiotoxicity (5). Again, as shown in Figure 3, we were unable to demonstrate protection by either vitamin D or sildenafil alone or by the combination treatment.

As in the studies presented in Figure 1, neither 1,25-D3 nor sildenafil, either alone or in combination, was shown to protect H9c2 cardiomyoytes from radiation (Figure 4).

We also examined the impact of 1,25-D3, sildenafil and the combination of 1,25-D3 and sildenafil on sensitivity to paclitaxel in both H9c2 and HL-1 cardiomyocytes. As shown in Figures 5 and 6, again, neither approach was successful in interfering with the damaging effects of paclitaxel in these experimental cell models.

Given these observations, we became interested in the possibility that autophagy might have some involvement in the response to radiation in the cardiomyocytes, particularly as autophagy has been demonstrated in cardiomyocytes by Adriamycin (6). Figure 7 shows the conversion of LC3 (microtubule light chain protein) from form I to form II in H9c2 cardiomyocytes by the higher doses of ionizing radiation, an indication of autophagy. These findings are confirmed by the data presented in Figure 8.

These findings have led to subsequent work in cardiomyocytes as well as animal models where we have attempted to develop models of radiation induced cardiac damage in efforts to identify strategies that might be effective in cardioprotection. We now have evidence for a mouse model of radiation induced toxicity (that is delayed for between 5-6 months) and where initial studies strongly suggest cardioprotection by sildenafil.

KEY RESEARCH ACCOMPLISHMENTS:

Demonstration that neither 1,25-D3 nor silenafil, alone or in combination, can protect cardiomyocytes from either cancer chemotherapeutic drugs or ionizing radiation.

Development of a mouse model of radiation induced cardiac injury**.**

REPORTABLE OUTCOMES:

None, as yet

FUNDING APPLIED FOR BASED ON WORK SUPPORTED BY THIS GRANITE

We have recently applied for a DOD Idea award based on radiation induced cardiac damage.

CONCLUSION:

We conclude that neither vitamin D nor sildenafil are effective at protecting cardiomyocytes from the toxicity of either doxorubicin, paclitaxel or radiation. Studies have been initiated in both cardiomyocytes and mice to determine whether IL-1 might be an alternative approach for cardioprotection.

REFERENCES:

1. Carreca I, Balducci L. Cancer Chemotherapy in the Older Patient. Urol Oncol. 2009 Nov-Dec;27(6):633-42.

2. Zhao G, Simpson RU. Interaction between vitamin D receptor with caveolin-3 and regulation by 1,25-dihydroxyvitamin D3 in adult rat cardiomyocytes. J Steroid Biochem Mol Biol. 2010 Jul;121(1-2):159-63. Epub 2010 Mar 19.

3. Pilz S, Tomaschitz A, Drechsler C, Dekker JM, März W. Vitamin D deficiency and myocardial diseases. Mol Nutr Food Res. 2010 Aug;54(8):1103-13.

4. Fisher PW, Salloum F, Das A, Hyder H, Kukreja RC. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. Circulation. 2005 Apr 5;111(13):1601-10.

5. Spallarossa P, Altieri P, Aloi C, Garibaldi S, Barisione C, Ghigliotti G, Fugazza G, Barsotti A, Brunelli C. Doxorubicin induces senescence or apoptosis in rat neonatal cardiomyocytes by regulating the expression levels of the telomere binding factors 1 and 2. Am J Physiol Heart Circ Physiol. 2009 Dec;297(6):H2169-81. Epub 2009 Oct 2.

6. Lu L, Wu W, Yan J, Li X, Yu H, Yu X. Adriamycin-induced autophagic cardiomyocyte death plays a pathogenic role in a rat model of heart failure. Int J Cardiol. 2009 May 1;134(1):82-90. Epub 2008 Jul 11.

APPENDICES: None

SUPPORTING DATA

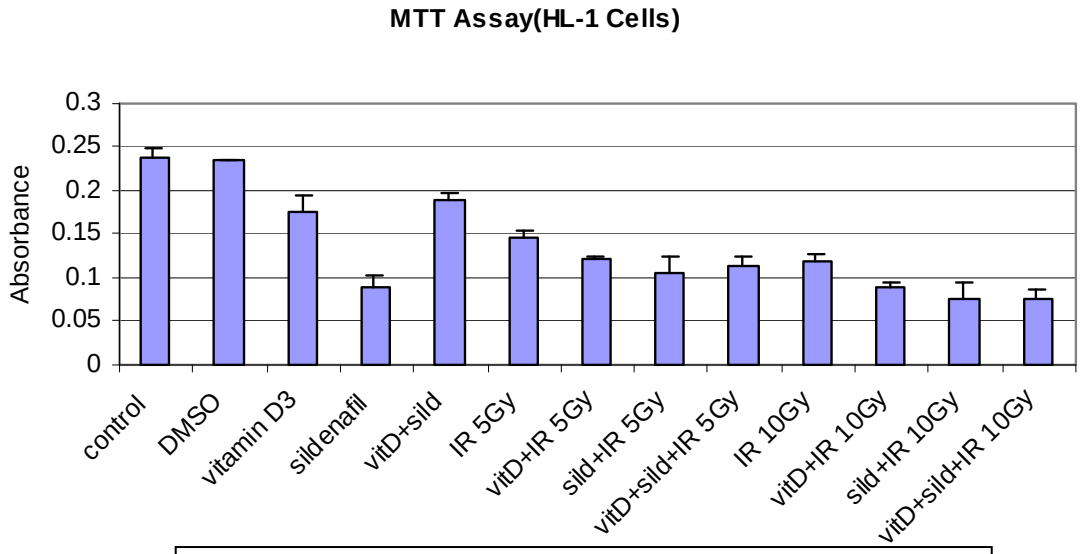


Figure 1. Effect of 1, 25-D3 alone (100nM), sildenafil (10uM) alone and the combination of 1,25-D3 + sildenafil on sensitivity to radiation in HL-1 cardiomyocytes. Sensitivity was determined using the MTT assay and is presented as relative absorbance.

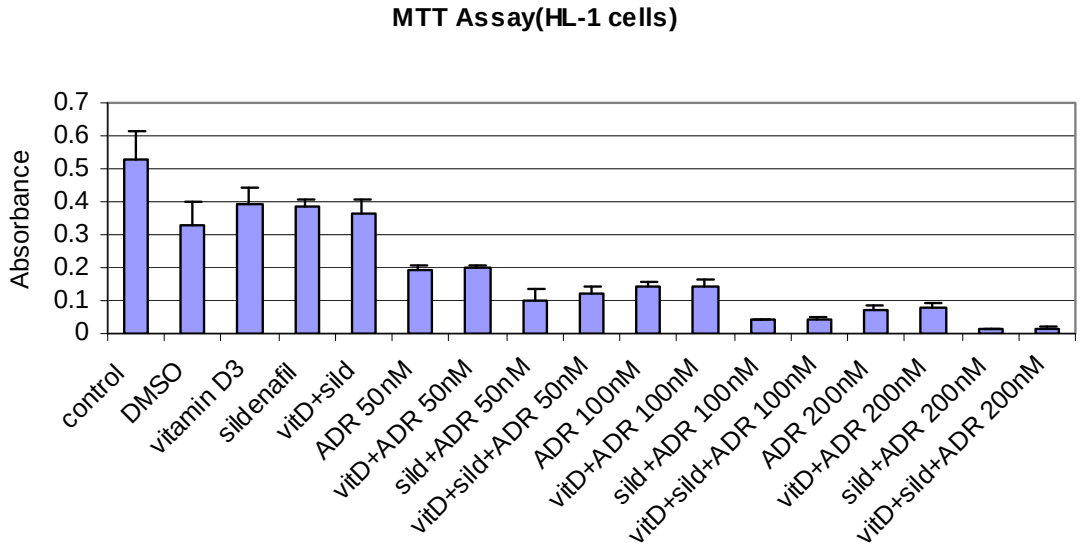


Figure 2. Effect of 1, 25-D3 alone (100nM), sildenafil (10uM) alone and the combination of 1,25-D3 + sildenafil on sensitivity to adriamycin (doxorubicin) in HL-1 cardiomyocytes. Sensitivity was determined using the MTT assay and is presented as relative absorbance.

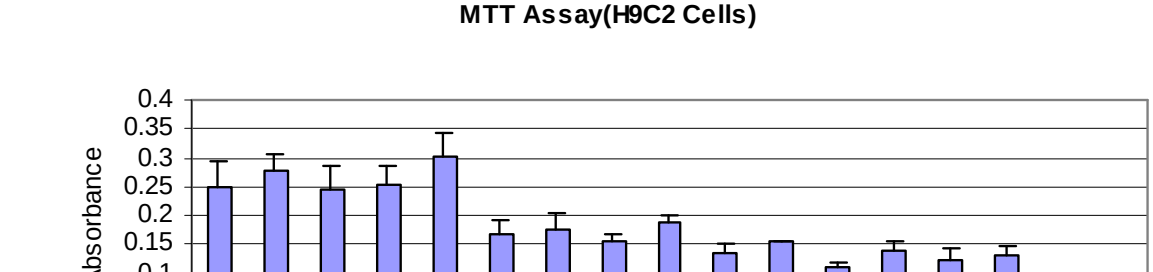


Figure 3. Effect of 1, 25-D3 alone (100nM), sildenafil (10uM) alone and the combination of 1,25-D3 + sildenafil on sensitivity to adriamycin (doxorubicin) in H9c2 cardiomyocytes. Sensitivity was determined using the MTT assay and is presented as relative absorbance

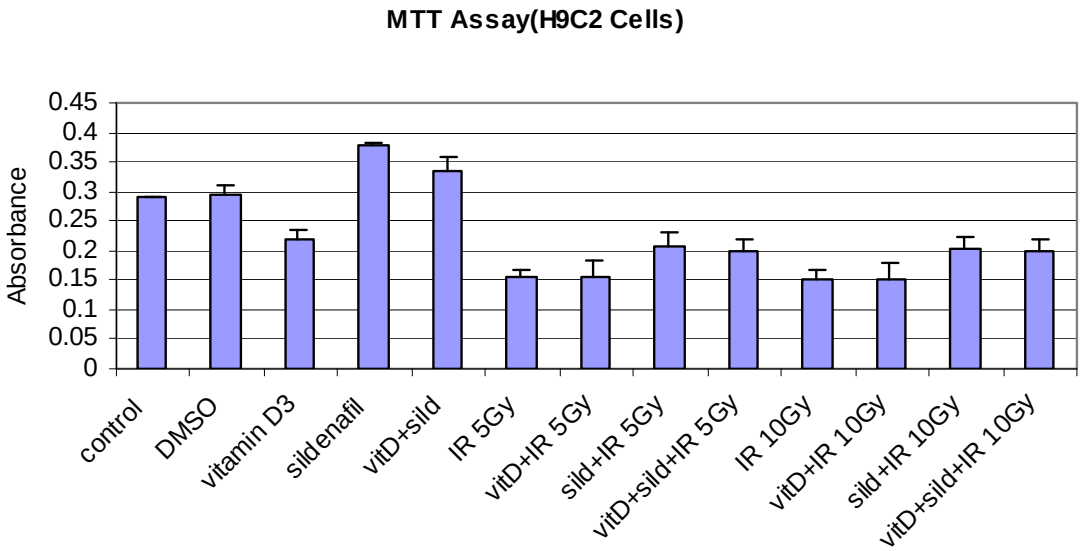


Figure 4. Effect of 1, 25-D3 alone (100nM), sildenafil (10uM) alone and the combination of 1,25-D3 + sildenafil on sensitivity to radiation in H9c2 cardiomyocytes. Sensitivity was determined using the MTT assay and is presented as relative absorbance.

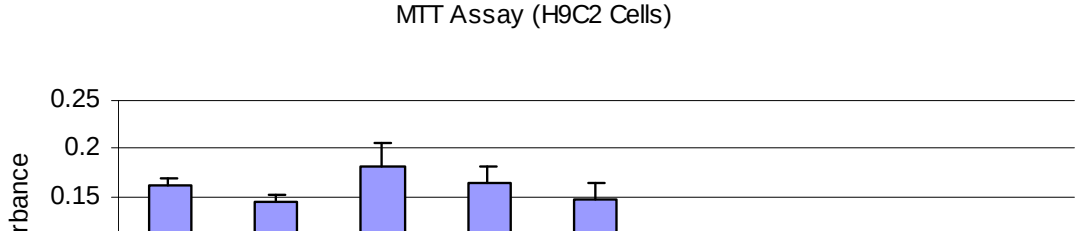


Figure 5. Effect of 1, 25-D3 alone (100nM), sildenafil (10uM) alone and the combination of 1,25-D3 + sildenafil on sensitivity to paclitaxel in H9c2 cardiomyocytes. Sensitivity was determined using the MTT assay and is presented as relative absorbance.

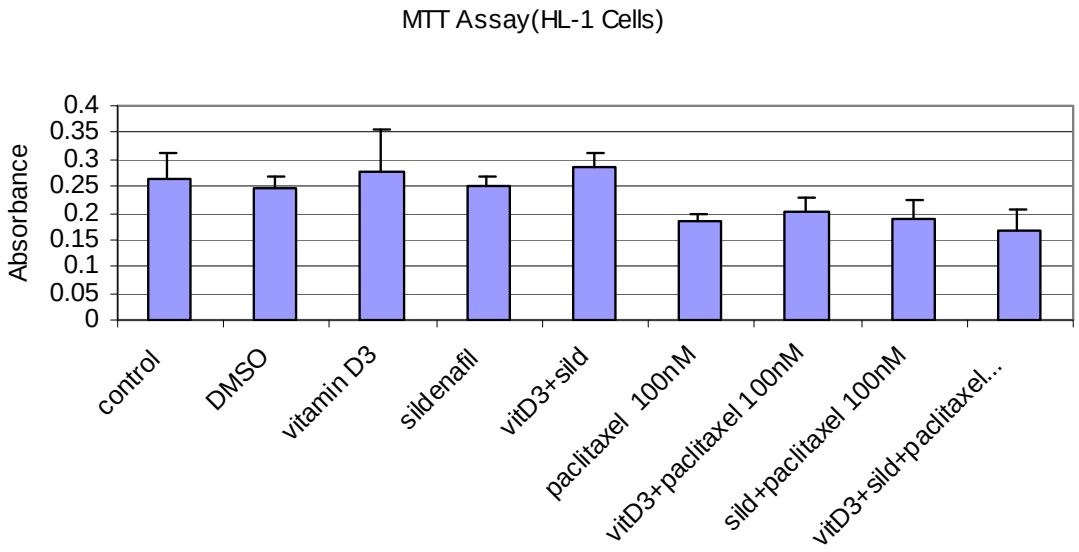


Figure 6. Effect of 1, 25-D3 alone (100nM), sildenafil (10uM) alone and the combination of 1,25-D3 + sildenafil on sensitivity to paclitaxel in HL1 cardiomyocytes. Sensitivity was determined using the MTT assay and is presented as relative absorbance

IR 0 2 5 10 Gy

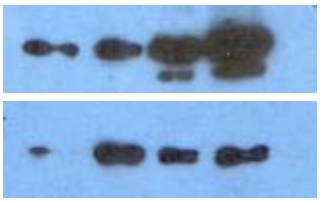
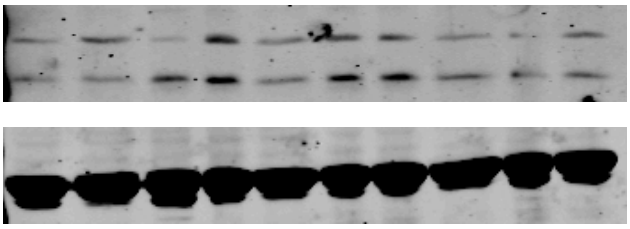


Figure 7. Conversion of LC3 (Form I) to LC3 (Form II) as an indication of autophagy in H9c2 cardiomyocytes



1. control
2. 2Gy 24h
3. 2Gy 48h
4. 2Gy 72h
5. 5Gy 24h
6. 5Gy 48h
7. 5Gy 72h
8. 10Gy 24h
9. 10Gy 48h
10. 10Gy 72h

Figure 8. Conversion of LC3 (Form I) to LC3 (Form II) as an indication of autophagy in H9c2 cardiomyocytes